as a mere accident of the moment. Such a phenomenon suggests the possibility of a rhythmic *a-v* association of a type not yet worked out and one the existence of which must be established by further observations before its etiology can be taken into account.

The prolonged condition of bradycardia and heart-block without convulsions or faints is the feature of Case III most deserving of emphasis.

I gratefully acknowledge the assistance of Dr. Theodore C. Janeway at every part of this study: in supplying the clinical material and polygraph, in the labor of recording the tracings, and in the many valuable suggestions he has given.

THE PHYSIOLOGY AND PATHOLOGY OF CREATININE AND CREATINE.

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Our knowledge of creatinine and creatine has reached a point where a résumé can with advantage be given to the clinician, and it is the purpose of this paper to present briefly the present conception of this subject.

With the appearance, in 1904, of the Folin[1] method for the accurate determination of both creatinine and creatine the investigation of the relation of these two bodies to metabolism received a great impetus. In his lecture on "Chemical Problems in Hospital Practice," delivered before the Harvey Society nearly two years ago, Professor Folin[2] made this statement: "I venture to predict that we shall learn more concerning the abnormal or subnormal metabolism of the sick on the basis of creatinine and creatine determinations alone than could be learned in another thirty years by means of the nitrogen determinations of the past." The importance and widespread interest in this question is again exemplified by the admirable paper of Professor Mendel,[3] given in the symposium on this subject before the various allied societies at their annual meeting in Baltimore last year.

The Physiological Elimination of Creatinine. Folin[4] was the first investigator to point out that the amount of creatinine excreted in the urine by a normal individual is quite independent of either the amount of protein in the food or of the total nitrogen in the urine, the amount excreted from day to day being practically constant for each individual (presumably in the same physical condition). The constancy of this creatinine excretion has been fully confirmed by van Hoogenhuyze and Verploegh,[5] Klercker,[6] Closson,[7] and Shaffer.[8] Shaffer[9] has further observed just as great uniformity in the hourly excretion of creatinine as is found in the daily excretion. Even very great diuresis has no effect on this hourly elimination. A great increase or decrease in the amount of protein ingested at a single meal, resulting in marked change in the amount of total nitrogen excreted per hour, is also without effect upon the hourly excretion of creatinine. Furthermore, experiments by both van Hoogenhuyze and Verploegh,[10] and Shaffer have shown that neither increased nor decreased muscular activity uncomplicated by other factors has any effect upon the creatinine elimination. Such results indicate that the regularity of excretion of creatinine is to be explained by a regularity of formation, and not merely by a regular kidney secretion.

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[1] Ztschr. f. phys. Chem., 1904, xli, 233.

[2] Jour. Amer. Med. Assoc., 1908, l, 1391.

[3] Science, 1909, xxix, 584.

[4] Amer. Jour. Phys., 1905, xiii, 46. See also Amer. Jour. Insan., 1904, lx, 699, and lxi, 299.

While the creatinine excretion is practically constant from day to day for each healthy individual, different persons excrete different amounts, and Folin pointed out that "the chief factor determining the amount of creatinine eliminated appears to be the weight of the person." He further noted that the fatter the subject, the less creatinine is excreted per kilo of body weight and concluded from this that the amount of creatinine excreted depends primarily upon the mass of active protoplasmic tissue. This, however, is not the only factor of influence, and on account of the complexity of the subject it is difficult to point out the factors involved. This question was discussed at length by Benedict and me[11] from the study of a large number of cases in the Connecticut Hospital for the Insane. In the case of women, the same investigators found that the creatinine excreted was, in general, much lower than that of men, doubtless due to their poorer muscular development. Here the creatinine-forming process is less active, and there is, at the same time, a lower muscular efficiency.

The significant physiological fact to be borne in mind in regard to creatinine is the absolute constancy of its elimination, different for different individuals, but wholly independent of quantitative changes in the amount of nitrogen excreted, thus pointing conclusively to its endogenous origin. As Folin has indicated, creatinine is by far the most reliable index as to the amount of a certain kind of true tissue katabolism occurring daily in any given individual. It has been found convenient to express the daily creatinine elimination in milligrams of creatinine nitrogen per kilo of body weight, and this has been called the creatinine co-efficient. For a strictly normal individual, this co-efficient is between 7 and 11 milligrams of creatinine-nitrogen.

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[5] Ztschr. f. phys. Chem., 1905, xlvi, 415.

[6] Beitr. z. chem. Phys. u. Path., 1906, viii, 59.

[7] Amer. Jour. Phys., 1906, xvi, 252.

[8] Ibid., 1908, xxiii, 1.

[9] Ibid., 1908, xxii, 445.

[10] Ztschr. f. phys. Chem., 1908, lviii, 161.

[11] Amer. Jour. Phys., 1907, xviii, 377.

THE ELIMINATION OF CREATININE IN PATHOLOGICAL CONDITIONS. That the creatinine elimination is affected by different pathological conditions has been shown by a number of investigators. In general, it may be stated that fevers produce an increase in its elimination, while a decrease is observed in a large number of other pathological states.

The effect of fever on the creatinine output has been studied by Leathes,[12] van Hoogenhuyze and Verploegh, Shaffer, and Klercker.[13] The rise in body temperature was found to be accompanied by an increased creatinine elimination. Van Hoogenhuyze and Verploegh noted the maximum creatinine excretion at the same time, or within a few hours of the highest temperature. In a study of such fevers as pneumonia, erysipelas, and typhoid, Klercker observed that during a long course of fever the height of the creatinine elimination decreases and may finally become considerably subnormal. After a short course of fever an increase may be found even during convalescence.

In an extended study of seven insane patients, van Hoogenhuyze and Verploegh observed variations in the creatinine elimination during periods of exultation and depression, there being an increase during the former and a decrease during the latter. They also observed an increase under the effects of stimulation with alcohol, syrup of cola, and strychnine, and a decrease following potassium bromide and absolute rest.

A low creatinine elimination has been found associated with a large number of abnormal conditions, especially those accompanied by muscular weakness. Benedict and I observed a creatinine co-efficient as low as 2 in two very old, decrepit women. In very young infants, Amberg and Morrill[14] found the co-efficient between 1.5 and 2.6, figures which one would expect from the small bulk of of muscular tissue. Funaro[15] obtained similar results.

In patients suffering from exophthalmic goitre Shaffer observed a very low excretion of creatinine, even though the total endogenous metabolism was above the normal, as shown by a rapid loss of weight and by emaciation. He concluded that creatinine was not a product of total tissue katabolism, but rather of certain normal cell processes, which in many diseased conditions may be extremely sluggish in their intensity. Forschbach[16] likewise observed a low creatinine output in cases of exophthalmic goitre. In two cases of myelogenous leukemia this author noted a low creatinine elimination, and in one case of lymphatic leukemia Shaffer reported a similar result. Mellanby[17] has recorded a diminished elimination of creatinine in disease of the liver, especially carcinoma.

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[12] Jour. Phys., 1907, xxxv, 205.

[13] Ztschr. f. klin. Med., 1909, lxviii, 22.

[14] Jour. Biol. Chem., 1907, iii, 311.

[15] Biochem. Ztschr., 1908, x, 467.

[16] Arch. f. exp. Path. u. Pharm., 1907, lviii, 112.

Spriggs[18] reported a very low creatinine excretion in two cases of muscular dystrophy (co-efficients 2.2 and 4.0), and in one case of amytonia congenita (co-efficient 1.9). He has also observed a much diminished elimination of creatinine in a case of pseudohypertrophic muscular dystrophy.[19] Levene and Kristeller[20] found very low creatinine co-efficients in four cases of muscular dystrophy, the co-efficient obtained from the average of six observations on one patient, aged seventeen years, being 1.5.

The effect of fasting on the creatinine elimination has been studied by van Hoogenhuyze and Verploegh, Benedict,[21] Benedict and Diefendorf,[22] and Cathcart.[23] In general, a moderate diminution in the creatinine has been noted during the progress of the fast. Similar results have been obtained on animals by Underhill and Kleiner[24] and also in experiments by Mendel and me.[25]

Changes have been noted in the creatinine elimination of experimental animals under the influence of various poisons, especially those which tend to produce a degeneration of liver tissue, such as potassium cyanide, hydrazine, phosphorus, amyl alcohol, and chromates. Such experiments have been performed by Loewy, Wolf and Osterberg,[26] Richards and Wallace,[27] Underhill and Kleiner, Lusk,[28] and Lefmann.[29]

Creatinine in Muscle. The recent papers of Grindley and Woods,[30] Mellanby, and Mendel and Leavenworth[31] appear conclusive that creatinine is not present preformed in the muscular tissue and hence our consideration of this compound is wholly confined to its elimination in the urine.

The Elimination of Creatine. In his original discussion of the subject Folin pointed out that although creatine is normally absent from urine, occasionally small amounts can be found. Benedict first noted that during inanition (experiments on a professional faster) considerable quantities of creatine appear in the urine, and subsequently in collaboration with Diefendorf confirmed this on a fasting woman. Independently, Cathcart observed this elimination of creatine during inanition. In fasting experiments which he conducted on rabbits, Dorner[32] also noted the appearance of creatine; while in similar experiments on dogs, Underhill and Kleiner, and Richards and Wallace obtained the same results. In an experiment made by Mendel and me on a fasting dog, it was found that after creatine had been continuously excreted for some time, it could be made to disappear by a practically pure carbohydrate diet.

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[17] Jour. Phys., 1908, xxxvi, 447.

[18] Quart. Jour. Med., 1907, i, 63.

[19] Biochem. Jour., 1907, ii, 206.

[20] Amer. Jour. Phys., 1909, xxiv, 45.

[21] Carnegie Inst., Washington, 1907, Pub. No. 77, 386.

[22] Amer. Jour. Phys., 1907, xviii, 362.

[23] Jour. Phys., 1907, xxxv, 500; also Biochem. Ztschr., 1907, vi, 133.

[24] Jour. Biol. Chem., 1908, iv, 165.

[25] Unpublished results.

[26] Biochem. Ztschr., 1908, viii, 123.

[27] Jour. Biol. Chem., 1908, iv, 179.

[28] Amer. Jour. Phys., 1907, xix, 464.

[29] Ztschr. f. phys. Chem., 1908, lvii, 476.

[30] Jour. Biol. Chem., 1907, ii, 309.

[31] Amer. Jour. Phys., 1908, xxi, 100.

[32] Ztschr. f. phys. Chem., 1907, lii, 225.

Benedict and I[33] were the first workers to report the elimination of creatine in a large number of individuals. In general, the quantity found was not large, though in one case the amount of creatine was greater than creatinine. The presence of this creatine in these cases may, perhaps, be generally explained as due to malnutrition.

Both Mellanby and van Hoogenhuyze and Verploegh have reported observations on patients suffering with carcinoma of the liver. Here the creatine elimination is very high, often a gram and a half per day. The latter investigators have found creatine almost absent in carcinoma of the stomach, thus pointing to some probable lack of liver function in the first instance.

In two cases of diabetes, Shaffer reports a considerable elimination of creatine, but in a measure, at least, this finds explanation in the meat diet. I have noted the same result in several cases which I have had occasion to examine. Shaffer has reported a considerable elimination of creatine in exophthalmic goitre. During the postpartum resolution of the uterus in women he has observed a marked elimination of creatine. This has also been noted by Murlin[34] in dogs. A considerable elimination of creatine during typhoid fever has been reported by Shaffer, and from a study of fevers Klercker concludes that sooner or later after the onset of fever, creatine is eliminated, a condition which lasts for a longer or shorter time, often to convalescence. Levene and Kristeller report the elimination of creatine, especially in their cases of muscular dystrophy.

Loewy, Wolf, and Osterberg, and Richards and Wallace, in their work with potassium cyanide, found the creatine elimination in dogs to be increased. Underhill and Kleiner obtained the same results with hydrazine, and likewise Lefmann from chromate poisoning.

CREATINE IN MUSCLE. According to Urano,[35] muscle tissue contains about 0.4 per cent. of creatine, and from this it has been calculated that a man weighing 60 kilos would have from 90 to 100 grams of creatine stored up in his muscle. In this connection, it is of interest that, according to Urano, the creatine of the muscle appears to be held in some non-diffusible form in the contractile tissue, and is only released when the integrity of the muscle bundle is impaired. The experiments of Mellanby indicate that the performance of muscular work leaves the creatine unaffected. Saiki[36] has demonstrated the presence of creatine in non-striated mammalian muscle, though here the quantity is much smaller, less than 0.1 per cent. It is not found, however, in invertebrate muscle.

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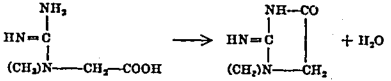
[33] Amer. Jour. Phys., 1907, xviii, 407.

[34] Ibid., 1909, xxiii, xxxi.

[35] Beitr. z. chem. Phys. u. Pathol., 1907, ix, 104.

RELATIONSHIP BETWEEN CREATININE AND CREATINE. Since creatine can be easily converted into creatinine under the hydrolytic action of weak acids, it was quite logical for the physiologist to assume some genetic relation between these compounds in the living body.



Creatine Creatinine

From this point of view, creatine was looked upon as a product of protein metabolism in muscle, easily converted into the "anhydride" creatinine and thus eliminated in the urine. However, the attempt to connect the excreted creatinine with tissue creatine brought to light an apparent independence of these compounds in metabolism. Ingestion of creatinine results in an increased creatinine elimination, but when creatine is fed to man or animals Folin,[37] Klercker,[38] van Hoogenhuyze and Verploegh, and Lefmann have all found that the creatinine content of the urine is only slightly altered if at all; nor is the creatine itself eliminated. Observations by Lefmann also uphold this for creatine introduced parenterally. The fact that ingested creatine is apparently not changed to creatinine in the body, together with the appearance of creatine in the urine during inanition and also certain other pathological conditions, led Folin, Benedict, and Klercker to question any biological relationship between these two compounds. The unique appearance of creatine under these circumstances must be borne in mind whenever this substance is found accompanying abnormal conditions attended by inadequate nutrition. In practically every such case it is reasonable to ascribe the source to the creatine of the muscle. As was stated above, creatine is excreted in acute fevers and various manifestations in which there is a rapid loss of muscle proteins. The creatine elimination is likewise marked in the urine of women during the first week post partum, when resolution of the uterus is taking place. However, in the majority of the conditions in which creatine is eliminated the creatinine excretion is abnormal and, as a rule, subnormal. This seems to be especially noteworthy in cases of muscular dystrophy and carcinoma of the liver. The evidence

[36] Jour. Biol. Chem., 1908, iv, 483.

[37] Festschrift f. Olof Hammarsten, 1906, III.

[38] Biochem. Ztschr., 1907, iii, 43.

presented by Mellanby, van Hoogenhuyze and Verploegh, and Lefmann, all point to a possible liver function in creatine and creatinine metabolism.

By an elaborate investigation of the behavior of these compounds during autolysis, Gottlieb and Stangassinger[39] have concluded that they can undergo a series of enzymatic transformations in the body, the more important of which are that, creatine can be formed in the autolysis of muscles and other organs, that preformed or added creatine can be converted by enzymatic means into creatinine during autolysis, and further, that both of these compounds may be destroyed by appropriate enzymes, creatase and creatinase. This was disputed by Mellanby, but after a repetition of the above work van Hoogenhuyze and Verploegh, Rothmann,[40] and Lefmann, all maintain the essential importance of endo-enzymes in the metabolism of the compounds under discussion. Such being the case, the liver is very probably the organ in the body most concerned in this action, especially in the formation of creatinine. Furthermore, it may be that when the tissues are drawn upon for supplies, as in hunger or cachexia, the creatine liberated by the disintegrating muscle now escapes destruction, owing to the lowered efficiency of some katabolic organ, such as the liver, and is eliminated as such.

At the present time it is impossible to formulate any hypothesis of creatine and creatinine metabolism which will correspond with all the data at hand. Creatinine is, however, very evidently a product of normal katabolism, possibly of muscle tissue. It is formed as the result of certain normal cell processes and is an index of the amount of a certain kind of true tissue metabolism. Creatine, on the other hand, is formed as the result of certain abnormal processes, and is, perhaps, an index of the amount of protein destruction. In one case the creatinine is apparently the result of the ordinary wear and tear, while in the other conditions, in which the protein is used to supply abnormal demands made upon it (energy, etc.), creatine is liberated by the disintegrating tissue. The experiments of Mendel and myself, in which carbohydrate was found to cause the urinary creatine of a fasting dog to disappear, harmonizes with this view. This hypothesis would explain why, in pathological cases, the total creatinine (creatinine + creatine) is not constant, though generally in the cases in which creatine is eliminated creatinine is diminished. (In fevers, the creatinine elimination is increased, due possibly to the high temperature.) Flesh destruction may be rapid, as in advanced carcinoma of the liver. In the tissues where this abnormal katabolism is taking place, creatine would result, while creatinine would be formed by the tissues which were functionating normally. If the tissue destruction were great, the creatinine would necessarily be low, and, on the other hand, the creatine would be high with a high total. According to the above idea creatinine and creatine would be two phases in the katabolism of some one substance, the one a product of normal katabolism, the other of abnormal katabolism.

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[39] Ztschr. f. physiol. Chem., 1907, lii, 1; ibid., 1908, lv, 322; and Stangassinger, ibid., 1908, lv, 295.

[40] Ibid., 1908, lvii, 131.

Many objections can be offered to such an hypothesis as the foregoing, but nevertheless it corresponds with much of the data at hand, and furthermore, aids in the interpretation of the results from a clinical standpoint.

THE METHOD OF DETERMINING CREATININE AND CREATINE. Inasmuch as the impetus for the recent work on creatinine and creatine has been due to the development of a satisfactory method for their accurate determination by Folin, a few statements can with advantage be made in regard to the method. This method is based upon the characteristic property possessed by creatinine of yielding a certain definite color reaction with picric acid in alkaline solution, the Jaffé color test. When properly diluted, the color corresponds almost exactly with $\frac{N}{2}$ potassium bichromate solution which is used as a standard. By means of a good colorimeter, such as the Duboscq, the quantity of creatinine can be estimated with great accuracy. For the determination of creatine, Folin originally recommended heating 10 c.c. of urine with an equal volume of normal hydrochloric acid on the water bath for three hours to convert any creatine to creatinine. The colorometric estimation would then give the total creatinine, and by subtracting the preformed creatinine from the total, the creatine would be obtained in terms of creatinine. While working with Benedict,[41] I found that this hydrolytic action by which the creatine was changed to creatinine could be completed in one-half hour in an autoclave at 117° to 120° C., and this is the method of conversion now generally employed.

SUMMARY. The significant physiological fact to be borne in mind in regard to creatinine, is the absolute constancy of its elimination, different for different individuals, but wholly independent of the volume of the urine and the amount of nitrogen excreted. Creatinine is an index of some special process of normal metabolism taking place largely, if not entirely, in the muscles. The intensity of this process appears to be associated with the muscular strength of the individual. In pathological conditions, the creatinine excretion is usually low. From this point of view, the creatinine output may be looked upon, at least to a certain extent, as an index of the physical condition of the patient. The creatinine elimination appears to be especially low in conditions associated with muscular weakness or inefficiency. It is, however, slightly increased in acute fevers and here does not run parallel with the muscular strength.

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[41] Amer. Jour. Phys., 1907, xviii, 397.

In diseases in which the creatinine output is low, creatine is generally excreted. Creatine is not normally present in the urine, and its excretion may be regarded as pathological. Furthermore, it is usually found to be associated with a loss of muscle protein, thus indicating that the source of the endogenous urinary creatine is the creatine of muscle tissue.

A SKIN REACTION IN CARCINOMA FROM THE SUBCUTANEOUS INJECTION OF HUMAN RED BLOOD CELLS.

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Clinical and experimental investigations have shown that in the growth and breaking down of malignant tumors substances are formed and set free which are very poisonous to the red cells of the blood, and which probably cause the anemia and cachexia of malignant disease. Although the nature of most of these poisonous substances has not yet been determined, some of them are known to belong to the class of lysins.

The knowledge that extracts of malignant tumors have marked hemolytic qualities naturally led to the search for hemolysins in the serum of animals and of man affected with malignant disease. If the blood serum of patients suffering from malignant disease contained hemolysins, while that of normal individuals or of those suffering from other diseases did not, then we would have at our command a method of value for the diagnosis of malignant disease. Crile, Weil, Pesskind, Blumgarten, Epstein and Ottenberg, Kelling, Kullman, and many others have made studies of the blood serum of patients, in order to determine whether there was anything characteristic in the blood of individuals with carcinoma. Crile claimed that in the early stages of carcinoma the blood serum was markedly hemolytic, and that as the disease advanced, the hemolytic properties of the serum disappeared. The other investigators substantiated Crile's claim that a number of the serums from carcinoma patients possessed hemolytic qualities, but they were able to find hemolytic serums only in a much smaller percentage of patients than did the first-named investigator. All agree that hemolysins for human red blood cells are present in 50 to 60 per cent. of the patients, although hemolysins exist in the serum of many other diseased individuals. The hope that the hemolytic test might prove of clinical value in the diagnosis of carcinoma has thus far been proved futile.

Weil, who was one of the earliest to investigate the subject, and who has published careful and critical studies of hemolysis in malignant disease, comes to the conclusion that it is possible that "something of value may eventually come of the use of this method in human disease, but that it will have to come with a refinement of method which will correspond to the complexity of the factors involved."

The hemolytic tests are made by mixing the serum of the patient with stated quantities of suspensions of washed human red blood cells from normal individuals. The tubes are kept in the thermostat for a number of hours, and the presence or absence of hemolysis is determined by the appearance of the supernatant fluid after the red cells have settled. If hemolysis has taken place, the fluid is colored pink or red by the hemoglobin which has been set free. By this method, the degree and presence of hemolysis is determined by the amount of laking of the red cells. The hemoglobin is therefore the indicator. The test-tube reaction gives no evidence of other substances which have been liberated. A small amount of hemolysin in the serum that is being tested may be insufficient to hemolyze enough cells to produce a coloration of the supernatant fluid appreciable to the eye.

It occurred to one of us[1] that it might be possible to gain a better knowledge of the hemolytic properties of the serum of human beings with malignant disease if red cells from a normal individual were brought into contact with the serum of patients *in vivo*—that is if the cells were injected under the skin of the patients.

Theoretically there might be certain advantages in such a method. If normal red blood cells are injected under the skin of a patient whose serum is hemolytic, fresh quantities of hemolysin would be continually brought to the cells by the circulating blood and lymph, and therefore—even if the amount of the hemolysin in the blood of the patient was very small—the injected cells might nevertheless be hemolyzed. Every organic substance which was liberated by the destruction of the injected red cells would enter the tissues and there have its effect. The hemoglobin and other substances which had been set free might cause a local change in the tissues at the site of the injection. If blood cells are injected under the skin of a normal individual, they are broken up and carried off by the body cells and fluids; the condition of affairs under the skin of a patient whose blood serum contains free hemolysins would be an entirely different one because a different process was taking place.

These theoretical considerations led one of us[2] to suggest that

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[1] Elsberg, Jour. Amer. Med. Assoc., March 27, 1909.

[2] Ibid.